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Acetyl-CoA Carboxylase—a Graminicide Target Site*

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Abstract: Acetyl-CoA carboxylase catalyses the first committed step in fatty acid (and acyl lipid) formation. The enzyme has been shown to exert a high degree of flux control for lipid biosynthesis in leaves and, therefore, it is not surprising that chemicals which can inhibit it effectively are successful herbicides. These chemicals belong mainly to the cyclohexanedione and aryloxyphenoxypropionate classes and are graminicides. The reason for the selectivity of these herbicides towards grasses lies in the nature of the target site, acetyl-CoA carboxylase.

Recent advances in our knowledge of acetyl-CoA carboxylases from sensitive and resistant plants has revealed some important facts. Dicotyledons, which are resistant, have a multi-enzyme complex type of carboxylase in their chloroplasts while grasses have a multifunctional protein. Both divisions of plants have two isoforms of the enzyme, the second being in the cytosol. Detailed study of multifunctional forms of acetyl-CoA carboxylases, which have different sensitivities to herbicides, suggests that herbicide resistance is correlated with cooperativity of herbicide binding to the native dimeric form of the carboxylase.

Key words: acetyl-CoA carboxylase, isoforms of acetyl-CoA carboxylase, graminicides, cyclohexanediones, aryloxyphenoxypropionates

1 INTRODUCTION

Acetyl-CoA carboxylase catalyses the first committed step in fatty acid synthesis. The enzyme is a Type I biotin-containing enzyme[1] which catalyses its reaction in two stages. First, there is a biotin carboxylation whereby there is an ATP-driven attachment of carbon dioxide from bicarbonate to the N^2-position of the biotin ring through the activity of biotin carboxylase. A carboxyphosphate intermediate is probably involved[1] and the reaction is stepwise. The prosthetic biotin group itself is attached to the so-called biotin carboxyl carrier protein (BCCP) through a lysine residue. Conserved sequences have been noted for the biotin attachment sites in a number of different biotin-containing enzymes. Moreover, the amino acid sequence in the vicinity of the biotin group provides considerable flexibility to the chain.[2] This flexibility and sequence characteristics are also shared by other enzymes where a prosthetic group has to interact with more than one active site during the course of the overall enzymatic reaction (e.g. pyruvate dehydrogenase).[1]

The second part of the acetyl-CoA carboxylase reaction is catalysed by a carboxyltransferase. This allows transfer of the carbon dioxide from biotin to acetyl-CoA to create malonyl-CoA. Although it is this part of the overall reaction that confers specificity on the individual biotin carboxylases, there is considerable amino acid sequence homology between the various carboxyltransferases.[3]

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TABLE 1
Evidence that acetyl-CoA carboxylase (ACCase) is important for regulating leaf lipid synthesis

1.	Analogy with animal ACCases.
2.	Reported coincidence of increased ACCase activity with increased lipid synthesis in seeds.
3.	Changes in pool sizes of acetyl- and malonyl-thioesters indicates strong control by ACCase during light-induced leaf lipid formation.
4.	Measurement of flux changes during leaf lipid synthesis shows that 50–60% of the total control is at the level of ACCase.

2 ACETYL-CoA CARBOXYLASE CONTROLS CARBON FLUX

Acetyl-CoA carboxylase has long been recognised as an important enzyme in controlling the rate of lipid synthesis in animals. Its activity in such organisms is controlled by differential gene expression, changes in protein degradation and by allosteric regulation.[2] Although it has been assumed, through analogy, that the plant enzyme would also be important, until recently evidence was only circumstantial. Recently, direct evidence that acetyl-CoA carboxylase is important in controlling light-driven leaf lipid synthesis has been obtained by measuring changes in the pools of thiolester intermediates.[4,5] This evidence has been strengthened considerably by measurements of flux control coefficients for acetyl-CoA carboxylase in barley and maize.[6] From flux control theory, the sum of the coefficients for all the individual enzyme-catalysed steps will equal 1. In the experiments with acetyl-CoA carboxylase, values of 0·5–0·6 were obtained. Bearing in mind that over twenty enzymes are involved in acyl lipid synthesis in leaves, these values (showing that up to 60% of the total flux control resides at the acetyl-CoA carboxylase step) emphasise the importance of acetyl-CoA carboxylase in regulating carbon flux to leaf lipids (Table 1). Such experimental results would give theoretical encouragement for the design of herbicides against the enzyme were it not for the fact that virtually all organisms need the enzyme and, therefore, such herbicides might be expected to be generally toxic.

3 THE NATURE OF PLANT ACETYL-CoA CARBOXYLASE

For over twenty years there has been considerable discussion as to the enzymatic nature of plant acetyl-CoA carboxylase. In the gram-negative bacterium *Escherichia coli*, separate proteins are present for the partial reactions and BCCP. These proteins associate to form a multi-enzyme complex. By contrast, in animals, acetyl-CoA carboxylase is a multifunctional protein with domains for the partial reactions and BCCP.[7] For plants, the first experiments to address the problem gave results which suggested that a multi-enzyme complex was present.[8] This was in spinach leaves. However, later work in other species clearly identified a high molecular mass (200–240 kDa) form of acetyl-CoA carboxylase[9] and it was assumed that proteinase activity gave rise to any smaller fragments observed. In the

TABLE 2
Some characteristics of acetyl-CoA carboxylase isoforms from the monocotyledon maize and the dicotyledon pea

Characteristic	*Isoform 1*	*Isoform 2*
Maize		
Molecular mass (kDa)	230	220
Subcellular Location	Plastid	Cytosol
Native structure	Dimer	Dimer
*K*m AcCoA (μM)	122 (±15)	113 (±12)
*K*m ATP (μM)	80 (±9)	76 (±8)
*K*m HCO_3^- (μM)	1091 (±242)	397 (±60)
I_{50} quizalofop (μM)	0·03	60
I_{50} fluazifop (μM)	10	1500
Pea		
Molecular masses (kDa)	32–79	230
Subcellular Location	Plastid	Cytosol
Native structure	Multi-enzyme complex	Dimer
*K*m AcCoA (μM)	250	15
*K*m ATP (μM)	250	170
*K*m HCO_3^- (μM)	870	2500
I_{50} quizalofop (μM)	>1000	7

Data from Herbert *et al.*[15] and Alban *et al.*[16]

last two years it has become clear that both types of enzyme are present with dicotyledons (such as spinach) containing both forms of acetyl-CoA carboxylase (see Table 2).

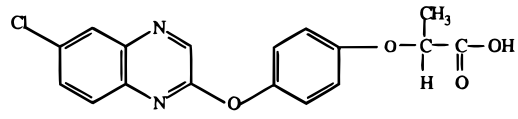
It has been known for some time that the product of the acetyl-CoA carboxylase reaction, malonyl-CoA, is required not only for the plastidial formation of fatty acids but also for many reactions taking place outside the chloroplast. For example, elongation of fatty acids for surface lipid formation[10] takes place on the endoplasmic reticulum and needs a source of malonyl-CoA from the cytosol. Because plastid-generated malonyl-CoA cannot cross the envelope membranes, a cytosolic location for acetyl-CoA carboxylase is clearly needed in addition to its being in the plastid. This suggestion was made and the possibility of isoforms proposed a few years ago.[11] Some preliminary evidence for isoforms was already available from protein purification[12] and herbicide studies.[13] Careful studies in several laboratories have now shown that in *Poaceae* there are two multifunctional forms of acetyl-CoA carboxylase[14,15] while in dicotyledons a multi-enzyme complex is present in chloroplasts and a cytosolic multi-functional acetyl-CoA carboxylase is also present.[16] Details of these enzymes are given in Table 2 and a fuller discussion has been presented by Harwood.[7]

4 HERBICIDE SENSITIVITY OF ACETYL-CoA CARBOXYLASE

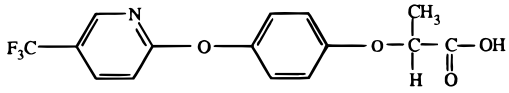
Two classes of chemical, the aryloxyphenoxypropionates (AOPPs) and the cyclohexanediones (CHDs) (Fig. 1) have proved to be very effective against *Poaceae*. Experiments by a number of laboratories showed that the target site was acetyl-CoA carboxylase and that the basis of selectivity for these graminicides lay in the structure of the protein. For a thorough discussion of this work see Refs 17–19.

AOPPs are applied as esters so that they can be absorbed effectively. They are then hydrolysed *in vivo* by carboxyl esterases and the free acid translocated to its site of action in the growing regions. Modification of the ester form can affect herbicidal activity and absence of the hydrolytic step can be a basis for resistance.[7] Because of an asymmetric carbon in the 2-substituted propionic acid moiety, the AOPPs exhibit stereoisomerism. Only the R(+) enantiomers have significantly activity against acetyl-CoA carboxylase[20] and this agrees with visual scoring of herbicidal damage.[21]

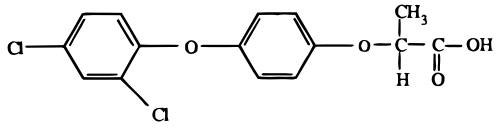
Apart from the commercially successful AOPPs and CHDs described earlier[17,18] some recently developed variations to the basic CHD structure have yielded very effective acetyl-CoA carboxylase inhibitors. However, these have yet to be tested at the whole plant level.[22] In addition, two other grass-selective compounds with structures different from AOPPs and CHDs have been reported.[7]



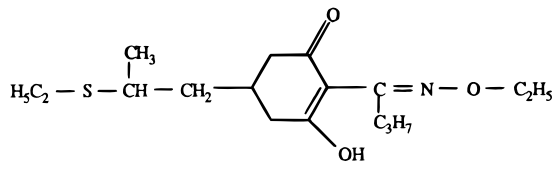
Quizalofop



Fluazifop



Diclofop



Sethoxydim

Fig. 1. Structures of some herbicidal aryloxyphenoxypropionic acids and the cyclohexanedione, sethoxydim.

The AOPPs and CHDs show linear, reversible inhibition against various acetyl-CoA carboxylases from susceptible grasses.[23,24] Kinetic studies, measurement of the partial reactions and the fact that other biotin-containing Type I carboxylases are not inhibited, showed that it was the carboxyltransferase partial reaction that was sensitive.[7] We have made a detailed study of the reaction kinetics and herbicide binding characteristics of the two isoforms of maize acetyl-CoA carboxylase.[25] Since these two isoforms differ greatly in their susceptibility to AOPPs or CHDs (see Table 2) then it is clearly of importance to try to explain their relative sensitivity. Their molecular masses, native conformations and Michaelis constants for the three substrates are all rather similar (Table 2). Moreover, the reaction characteristics were close to an ordered mechanism in both cases.[25] However, differences were noted in the binding characteristics towards quizalofop or fluazifop. The major isoform (isoform 1, located in the plastid) showed no significant cooperativity for binding. In contrast, isoform 2, which had reduced sensitivity to graminicides, exhibited strong positive cooperativity (Fig. 2).[25] Thus, binding of graminicide to one half of the native dimer changed its ability to bind to the second

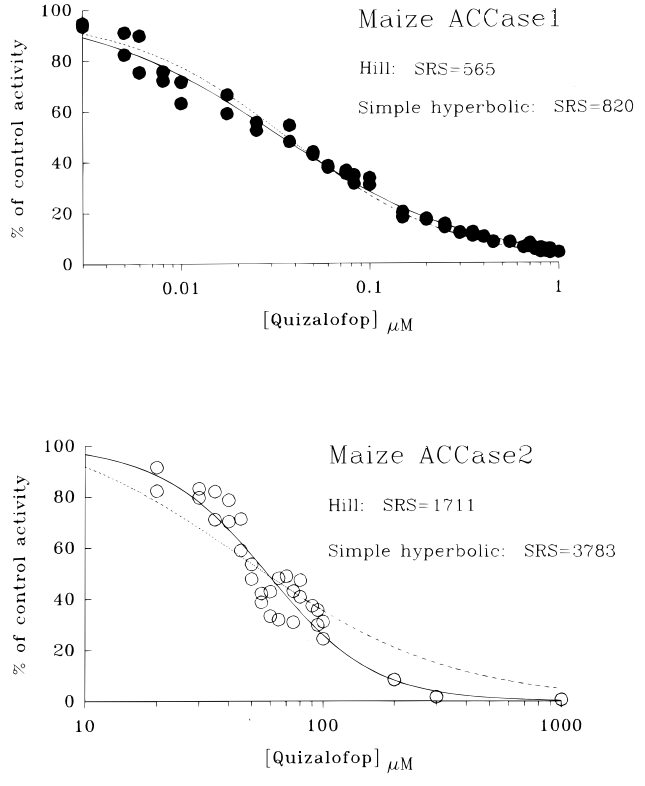


Fig. 2. Curve fits for (——) cooperative and (- - -) non-cooperative binding of quizalofop to maize acetyl-CoA carboxylase isoforms. SRS = sum of residual squares. Data taken from Ref. 26, with permission.

site. This characteristic for cooperativity of binding is also associated with less activity against propionyl-CoA (a partial reaction catalysed by acetyl-CoA carboxylase rather than by a separate propionyl-CoA carboxylase).[26] It seems possible that cooperativity of herbicide binding is a property of importance and is associated with insensitivity of the enzyme.

No study of the enzyme reaction for separated acetyl-CoA carboxylase isoforms from resistant grasses has been made. However, cooperativity of binding graminicides has been reported for two insensitive, mixed (two isoforms), acetyl-CoA carboxylase preparations, from *Poa annua* L.[26] and from *Lolium multiflorum*.[27] Significantly, acetyl-CoA carboxylase preparations from a susceptible biotype of *L. multiflorum* showed only weak cooperativity.[27] These results agree with our contention that cooperativity of herbicide-binding may be associated with acetyl-CoA carboxylase insensitivity.

Mention of insensitive acetyl-CoA carboxylases from grasses leads one to the increasing problem of herbicide resistance. Because the mechanism of selectivity lies in the nature of the target site protein, it is not surprising that resistance problems have arisen. In many cases, resistance is at the level of the acetyl-CoA carboxylase, presumably due to a point mutation.[28] In view of the demonstrated differences in herbicide-binding characteristics between sensitive and insensitive forms of acetyl-CoA carboxylase, it seems very important to evaluate these newly resistant grasses. Such studies may suggest possibilities for modifying graminicide structures in order to make both more effective pesticides as well as ones less prone to resistance problems.

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